E. coli infections in poultry - what do we know and where to go.

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E. coli infections in poultry - what do we know and where to go

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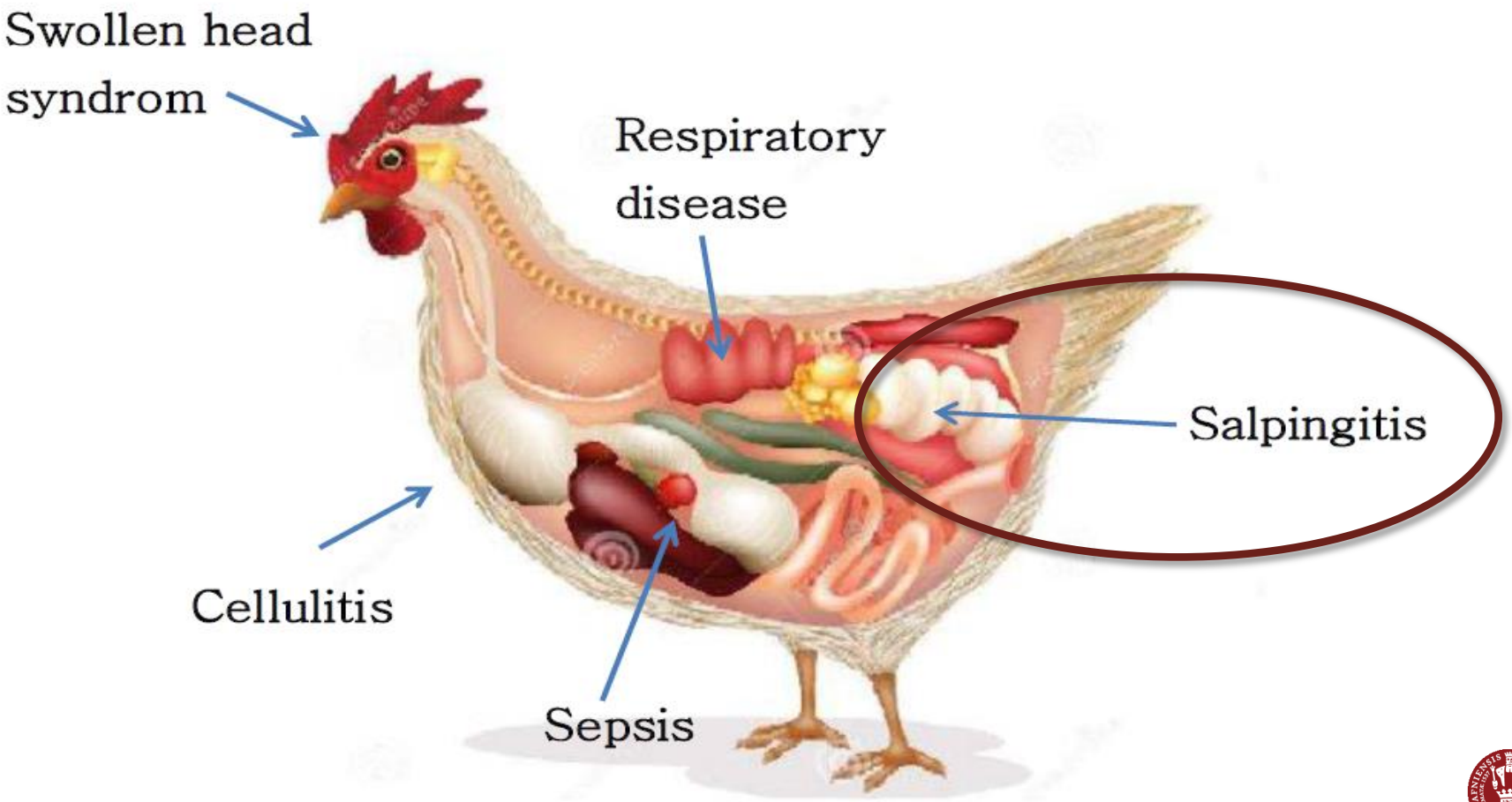
Avian Pathogenic *E. coli* (APEC)

Examples of disease mainfestations in poultry



What characterize an Avian Pathogenic *E. coli* (APEC)?

- Only focusing *in vitro* on the infections agent (*E. coli)* is not sufficient!

- *In vitro* data must be combined with

  - Flock data (Outbreak/single case?)
  - Host status (Age, vaccination, viral infection?)
  - Pathology (Infectious/non infections?)
  - Bacteriology (Other infections, pop. structure)

Aim

We aimed to achieve a greater insight into the nature of avian *E. coli* salpingitis combining

- Field outbreak study (bacteriology/pathology)
- Experimental challenge studies
  - Whole genome sequencing analysis of strains of different in vivo pathogenicity

Materials and methods
Flock data and bacterial isolation

- Inclusion criteria:
  Layer flocks with increased mortality due to "colibacillosis"

- Detailed post mortem (pm) examination of submitted layers

- Swabs obtained from liver, spleen and salpinx during pm

- Pure culture *E. coli* isolates further characterized

Materials and Methods – Field study of outbreak of *E. coli*

- 8 layers flocks with increased mortality due to *E. coli* (primarily salpingitis)
- Multilocus sequence typing[1]
- Phylogenic groups[2]
- Virulence genes (*astA*, *papC*, *iucD*, *cva/cvi*, *ompT*, *iroN*, F11) [3-5]

Will not be discussed today due to time

[1] Wirth et al. 2006, [2] Clermont et al. 2000, [3] Ewers et al (2005), [4] Johnson et al. 2006, 5 Johnson et al. 2000

Results – Overview

Outbreak number	Sub-mission	Age (wks)	No. of birds	Cause of mortality	No. of birds demonstrated abundant growth of *E. coli* in pure culture	Population structure of *E. coli*
A1	1	45	26	*E. coli* (13), cannibalism (13)	13 (50%)	Polyclonal
[illegible]	[illegible]	[illegible]	[illegible]	[illegible] (sterile) accompanied by amyloidosis (1)	[illegible]	[illegible]
C1	1	78	16	*E. coli* (11), *G. anatis,* (2), cannibalism (3)	11 (69%)	Polyclonal
D1	1	65	56	*E. coli* (43), *S. aureus* (1), cannibalism (11), killed by owner (1)	43 (77%)	Monoclona
D2	2	68	48	*E. coli* (29), chronic salpingitis accompanied be nephropathy (*E. faecalis*) (1), chronic salpingitis (sterile) accompanied by nephropathy (1), cannibalism (17)	29 (60%)	Monoclona
E1	1	75	42	*E. coli* (39), *G. anatis,* (1), *E. faecalis* (1), [illegible]	39 (93%)	Monoclona
F1	1	27	12	*E. coli* (12)	12 (100%)	Monoclona
F2	2	31	16	*E. coli* (15), cannibalism (1)	15 (94%)	Monoclona
G1	1	55	31	*E. coli* (11), cannibalism (6), emaciation, dehydration and nephropathy (1) histomoniosis (1), egg bound (1), amyloidosis(1), liver rupture (1), cellulitis (1), out of lay and killed by owner (2)	11 (35%)	Polyclonal
H1	1	75	43	*E. coli* (24), *Streptococcus pluranimalium (*1), cannibalism (12), uraemia (3), egg bound (3)	24 (56%)	Polyclonal
	10		**322**		**220 (68%)**	

Results

Field study - Multilocus sequence typing

ST	# isolates (n=100)	Prevalence in flocks	Comments
23	21	2	Mono- and polycl. outbreaks
79	4	2	Polycl.
95	33	2	Monoclonal
117	4		"Background" monoclonal"
140	17		Mono- and polycl. outbreaks
141	4		Polycl.
357	3		Polycl.
429	4		Polycl.
2490	2		Polycl
67,93,101,155,373,641	1/ ST	1-5	All except ST101 associated with polyclonal outbreaks

Want to know more?

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Pathology and Molecular Characterization of *Escherichia Coli* Associated With the Avian Salpingitis-Peritonitis Disease Syndrome

Author(s): Rikke Heidemann Olsen, Magne Bisgaard, Jens Peter Christensen, Susanne Kabell, and Henrik Christensen

Source: Avian Diseases, 60(1):1-7.

Published By: American Association of Avian Pathologists

DOI: http://dx.doi.org/10.1637/11237-071715-Reg.1

URL: http://www.bioone.org/doi/full/10.1637/11237-071715-Reg.1

Results

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67,93,101,155,373,641	1/ ST	1-5	All except ST101 associated with polyclonal outbreaks

Model of ascending infection developed;
Can Koch's postulate be fulfiled?

Materials and Methods – Experimental Challenge Study

- Surgical model[1]

  - 10 Chickens (25 wks)/group
  - Dose 0,1 ml $6*10^5$ CFU *E. coli*

- Challenge strains

Strain	ST	Phylo-group	Clinical information
Sept362	117	D	Isolated from a chicken with septicemia
SCI-07	369	B2	Isolated from a chicken with swollen head syndrom (SHS)
Chronic_Salp	429	B2	Isolated from a chicken with chronic salpingitis (not outbreak related)
Cp6Salp3	95	B2	Isolated from a chicken with severe salpingitis and sepsis (outbreak related)
D2-2	115	D	Commensal strain isolated from the cloacae of a healthy chicken

- Different infection susceptibility of layers vs. broiler breeders?

[1] Pors et al. 2014.

Result and discussion

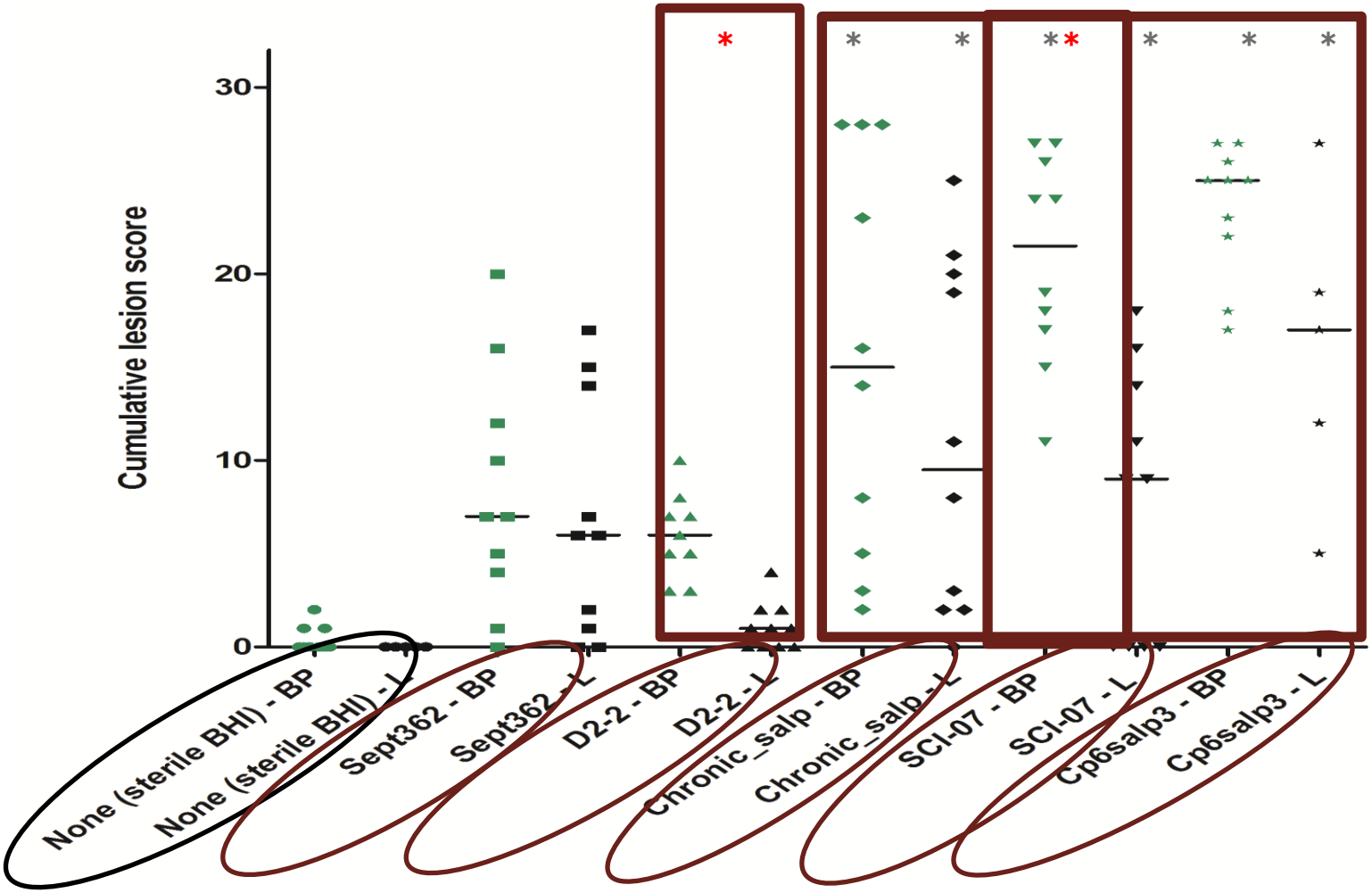
Clinical and pathological outcome

- From limited/none pathology to salpingitis/fibrino-purulent peritonitis/sepsis. Pathology highly similar to field outbreak pathology

Result and discussion

Clinical and pathological outcome

* Sign. Difference between L and BP
* Sign. Different from control



Result and discussion - Genomic comparison of challenge strains

Characteristics	Strains: Cp6Salp3	Chronic_salp	D2-2	SCI-07	Sept362	
Genome size (Mbp)	5,10	5,03	5,68	4,97	5,28	NCBI
Sequence type	95	429	115[1]	368[1]	117[1]	[1]MLST 1.7 [12]
Serotype	O1:K1:H7	083:H4	O2:H9	H31	0109:H10	SeroTypeFinder https://cge.cbs.dtu.dk/services/SeroTypeFinder/
Plasmids (replicon types)	IncFII IncFIB	IncI1, IncFII IncFIB	IncI1, Col8282, col1156, B/O/K/2	IncB, IncFII IncFIB, IncFIC,	IncI1,IncFII IncFIB, IncFIC, IncFIA	PlasmidFinder 1.2 [5]
No. predicted pathogen families	959*	623	523	471	478	Pathogen finder 1.1[10]
Virulence, Disease and Defense genes	109	109	117	124	118	SEED prediction [30]
Iron acquisition and metabolism	82	53	99	90	80	SEED prediction [30]
Phage associated genes	143*	95	116	79	91	SEED prediction[30]
CRISPR	2	1	2	3	2	CRISPRfinder [12].
Virulence profile[a]	Pap operon[b], vat, upaG,tia,irp2,tsh, iutA, iucA/B/C/D, ehaB, iroN, cva/cvi, maxI, hlyF	irp2, fuyA, tsh, ehaB, cva/cvi, maxI, hlyF	irp2, fuyA, iutA, iucA/B/C/D, iroN, eitB, maxI	irp2, fuyA, iutA, iucA/B/C/D, iroN, eitB, cva/cvi, maxI	tsh, iutA, iucA/B/C/D, iroN, eitB, cva/cvi, maxI	BRIG [12] (Sequences for comparison were obtained at VFDB [12])

- IncF

Want to know more?

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journal homepage: www.elsevier.com/locate/vetmic

Experimental induced avian *E. coli* salpingitis: Significant impact of strain and host factors on the clinical and pathological outcome

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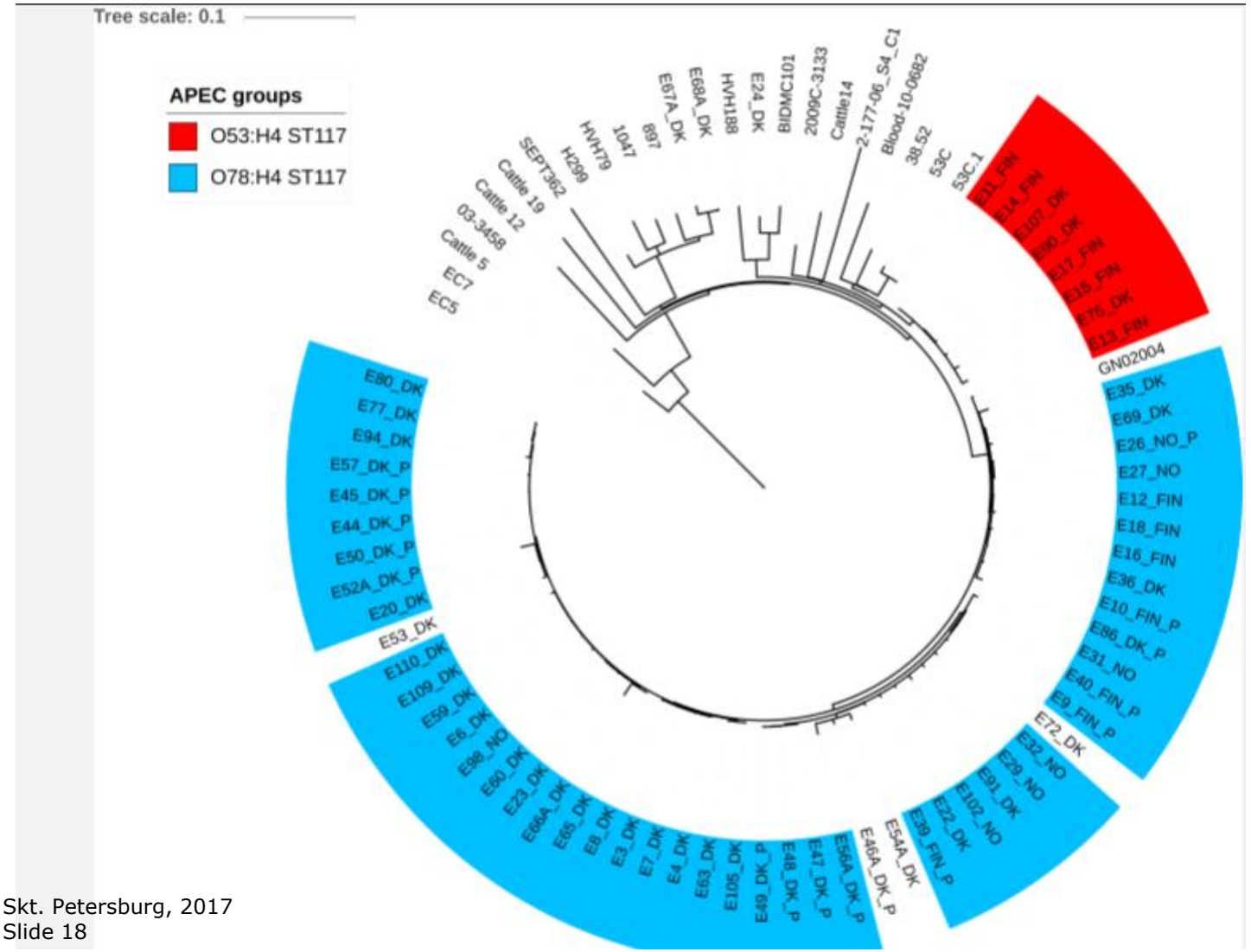
Sum up

- A broad diversity of *E. coli* strains are capable of causing ascending infection, but is highly depended on host factors
- High virulent clones causing monoclonal outbreaks in the field
- These high-virulent clones may represent true APEC, hence, not being opportunistic
- In general broiler breeders more susceptible than layers

E. coli challenges in the Scandinavian countries 2014-2016

Whole genome sequencing used to trace the source



Want to know more?

Ronco *et al. BMC Genomics* (2017) 18:13
DOI 10.1186/s12864-016-3415-6

BMC Genomics

RESEARCH ARTICLE Open Access

Spread of avian pathogenic *Escherichia coli* ST117 O78:H4 in Nordic broiler production

Troels Ronco[1*], Marc Stegger[2], Rikke Heidemann Olsen[3], Camilla Sekse[4], Anne Bang Nordstoga[4], Tarja Pohjanvirta[5], Berit Lilje[2], Ulrike Lyhs[1], Paal Skytt Andersen[2] and Karl Pedersen[1]

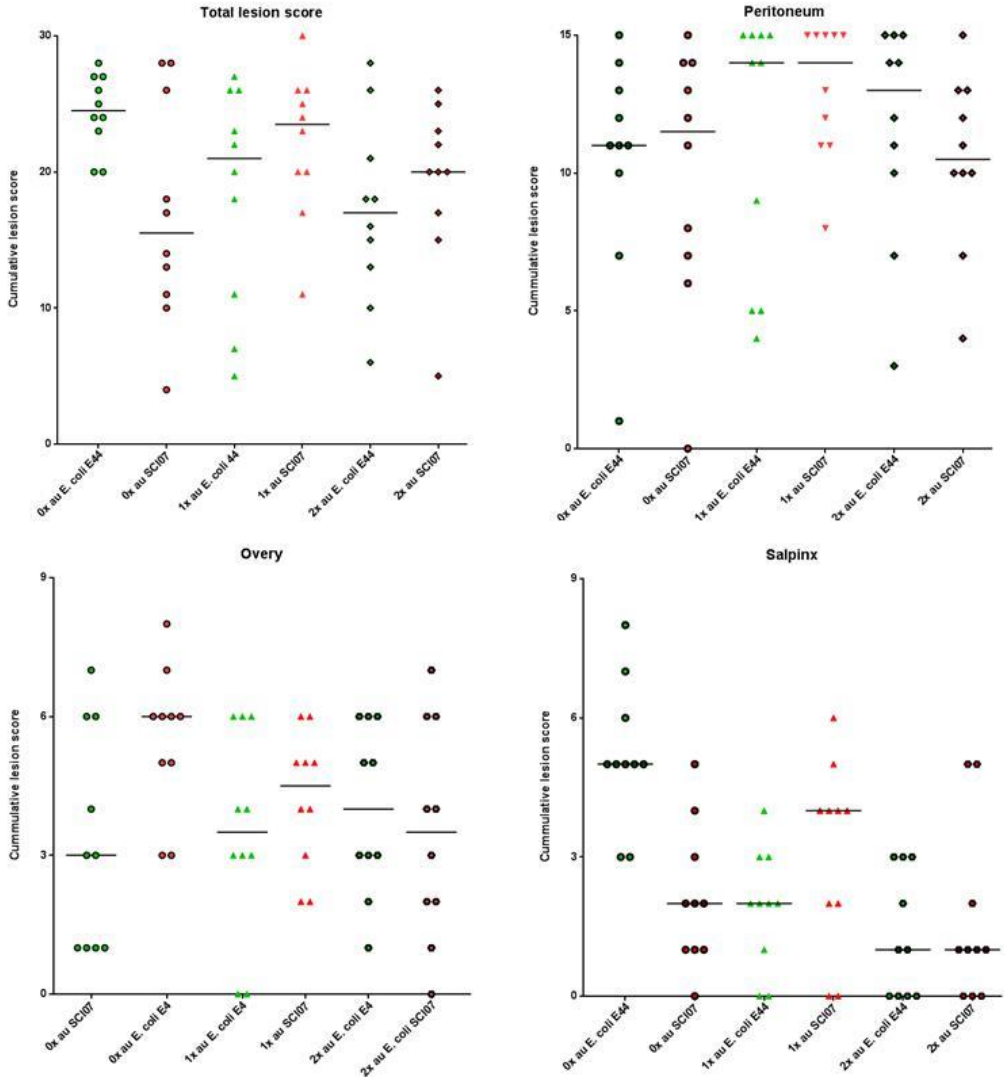
One particular clone of *E. coli* caused the majority of mortality

The Danish strategy: Autogenously vaccination (+poul vacc)

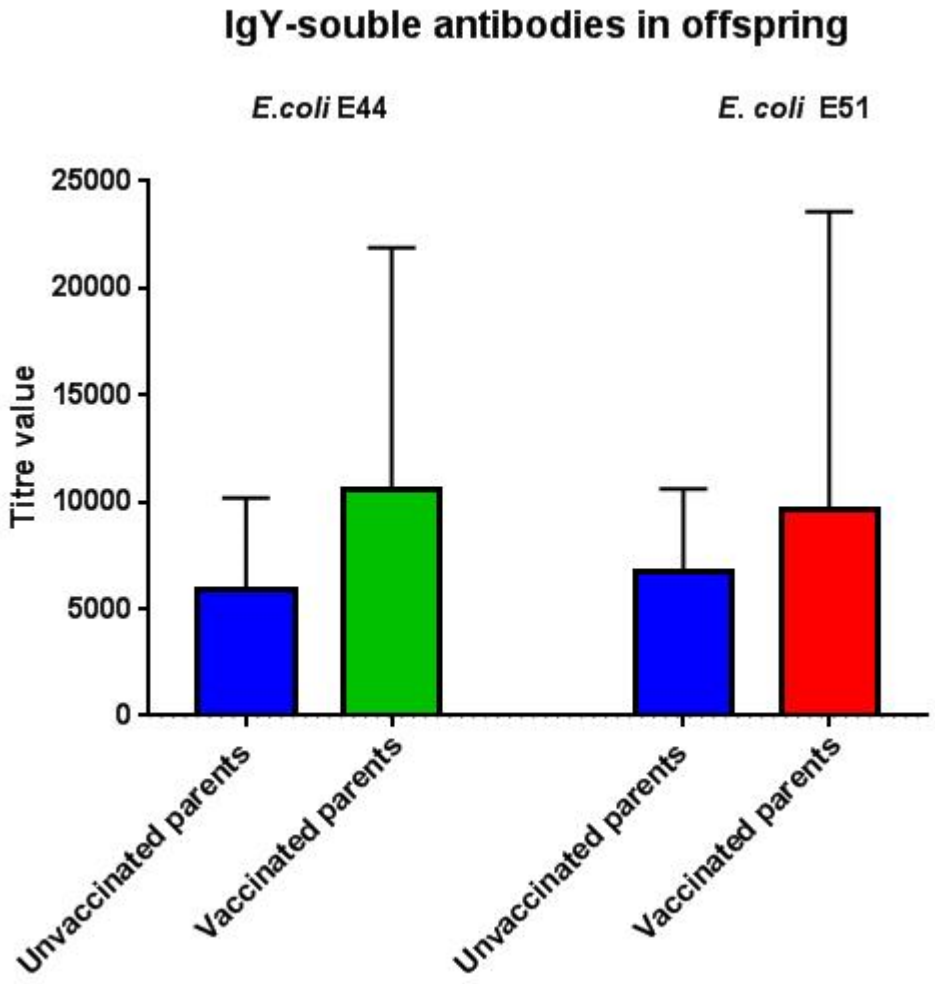
Experimentel set-up to test efficiantcy of autogenous vaccine

Group	E. coli autogenous vaccine (week of age)	Challenge strain[1]	Average weight at day of challenge	Survived the full experimental period (7 days post challenge)
1A	None	E. coli E44 **(autovaccine strain)**	3699	0/10 [a]
1B	None	SCI_07	3669	10/10
2A	1 time (18)	E. coli E44 **(autovaccine strain)**	3532	8/10
2B	1 time (18)	SCI_07	3529	10/10
3A	2 times (14 and 18)	E. coli E44 **(autovaccine strain)**	3563	9/10
3B	2 times (14 and 18)	SCI_07	3543	10/10

Similar pathology between vaccinated-unvaccinated



No difference in level of specific antibodies in the offspring



Want to know more?

Future perspective

- Accept ”normal *E. coli* mortality” (Optimize management/biosecurity)
- Focus on the **true APEC**
- The impact of vertical transmission of high-virulent clones of *E. coli* ?
- Target particular clones in a vaccine approach to decrease vertical/horizontal transmission

Thank you for your attention!

cava@sund.ku.dk

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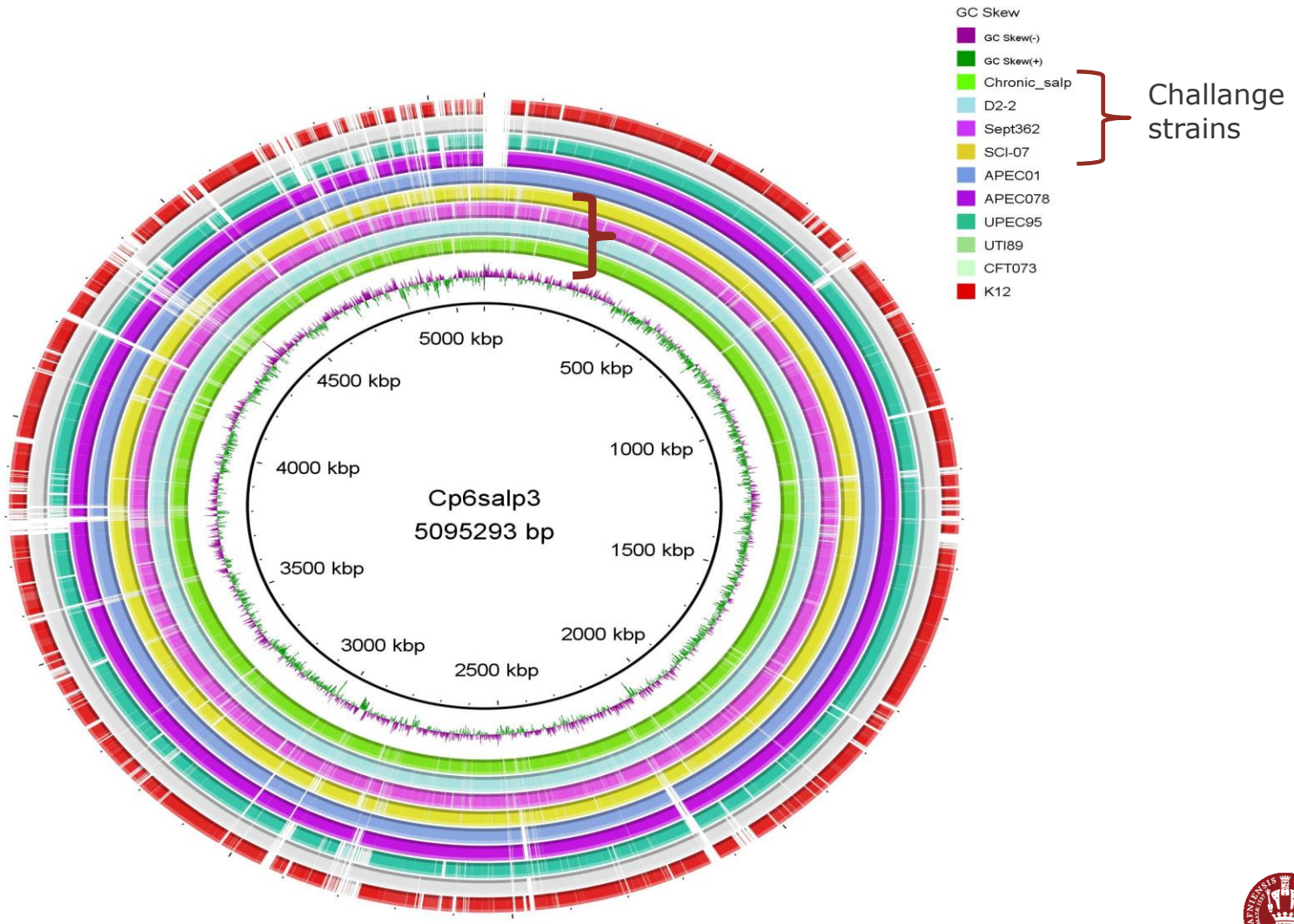
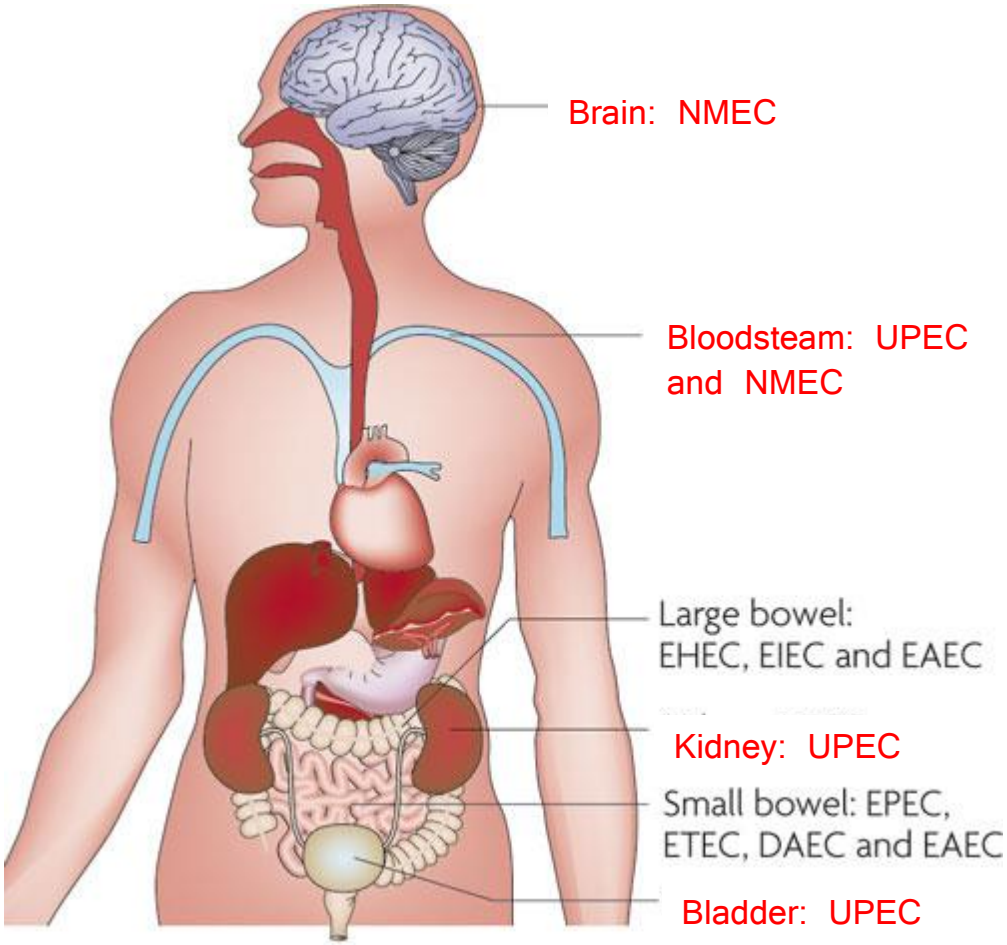
Result and discussion

Genomic comparison of challenge strains



Skt. Petersburg, 2017

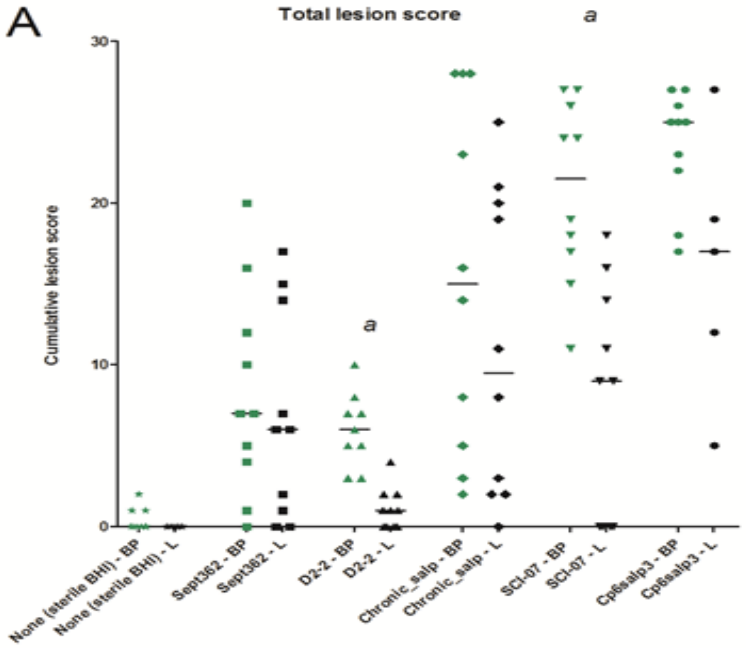
Pathotypes of human pathogenic *E.coli*

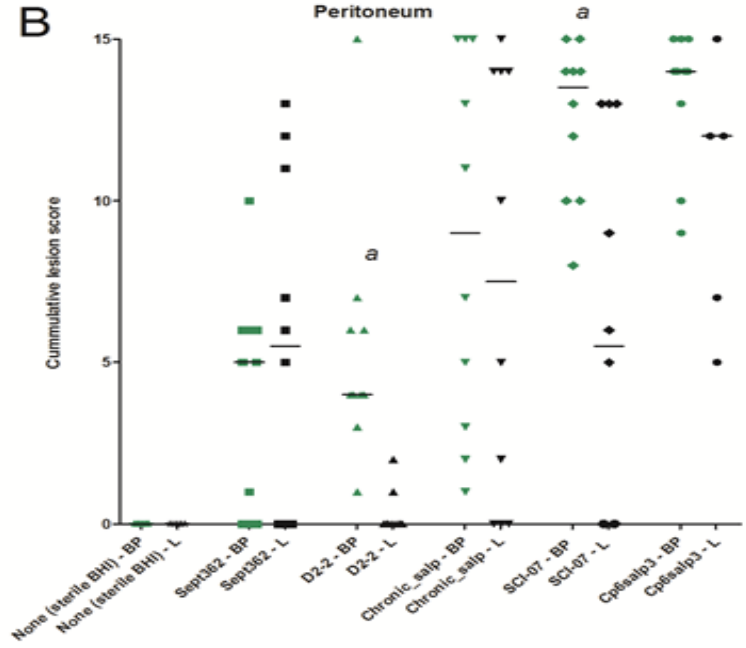


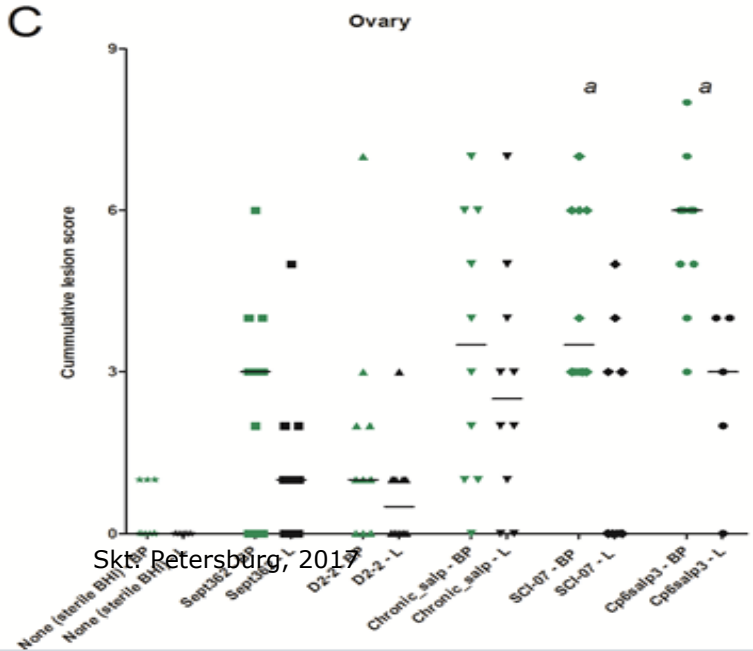
Croxen and Finlay (2010) Nature Reviews | Microbiology

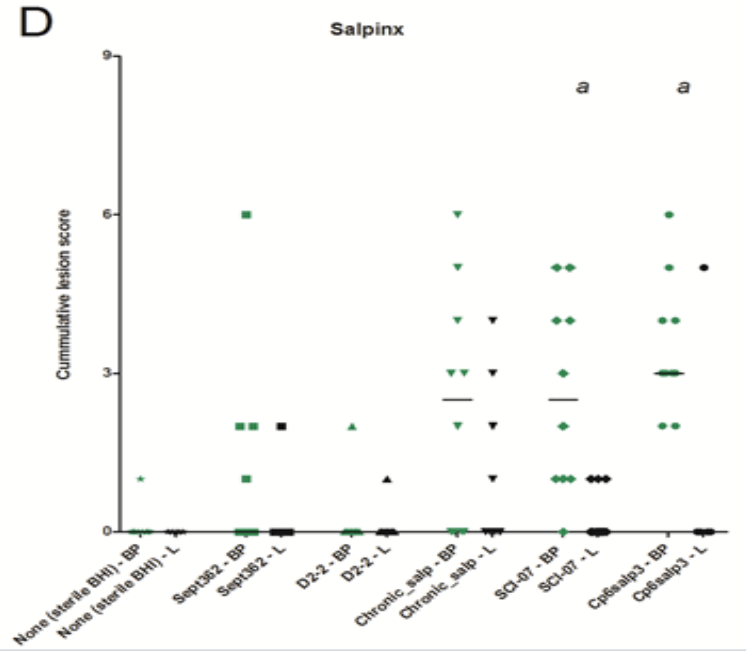
Definition of an *E. coli* pathotype (In human medicine)

"A defined pathological lesion caused by a population of *E. coli* having characteristic mechanisms to attach to and exploit the host's cells of a particular niche"


A
Total lesion score
a
Cumulative lesion score
30
20
10
0
None (sterile BHI) - BP
None (sterile BHI) - L
Sept362 - BP
Sept362 - L
D2-2 - BP
D2-2 - L
Chronic_salp - BP
Chronic_salp - L
SCI-07 - BP
SCI-07 - L
Cp6salp3 - BP
Cp6salp3 - L


B
Peritoneum
a
a
Cummulative lesion score
15
10
5
0
None (sterile BHI) - BP
None (sterile BHI) - L
Sept362 - BP
Sept362 - L
D2-2 - BP
D2-2 - L
Chronic_salp - BP
Chronic_salp - L
SCI-07 - BP
SCI-07 - L
Cp6salp3 - BP
Cp6salp3 - L


C
Ovary
a
a
Cummulative lesion score
9
6
3
0
None (sterile BHI) - BP
None (sterile BHI) - L
Sept362 - BP
Sept362 - L
D2-2 - BP
D2-2 - L
Chronic_salp - BP
Chronic_salp - L
SCI-07 - BP
SCI-07 - L
Cp6salp3 - BP
Cp6salp3 - L


D
Salpinx
a
a
Cummulative lesion score
9
6
3
0
None (sterile BHI) - BP
None (sterile BHI) - L
Sept362 - BP
Sept362 - L
D2-2 - BP
D2-2 - L
Chronic_salp - BP
Chronic_salp - L
SCI-07 - BP
SCI-07 - L
Cp6salp3 - BP
Cp6salp3 - L